

PRELIMINARY OBSERVATIONS ON THE REPRODUCTION, GROWTH AND DIET OF *UROLOPHUS CRUCIATUS* (LACÉPÈDE) AND *UROLOPHUS EXPANSUS*, McCULLOCH (*UROLOPHIDAE*) IN SOUTHEASTERN AUSTRALIA

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This study examines the diet, growth and reproduction of two species of stingarees (*Urolophus cruciatus* and *U. expansus*) that occur off the south-east coast of Australia and are bycatch of commercial trawlers targeting latchet, flathead and jackass morwong. Both stingarees prey on similar species but in different proportions. Dominant prey for both stingarees were primarily crustaceans (isopods) followed by polychaetes. No significant difference was found between sexes though diet varied with size in both species. Sexual maturity for female and male *U. cruciatus* was attained at 320 mm and 315 mm total length, respectively. Male *U. expansus* matured at a total length of 360 mm with insufficient numbers of females to determine sexual maturity.

Keywords: Urolophids, stingarees, von Bertalanffy, prey

THE family Urolophidae (round stingrays and stingarees) contains three genera (*Urolophus*, *Trygonoptera* and *Plesiobatis*) and 40 species, 22 of which occur in Australian waters. Stingarees are found in habitats ranging from shallow to inner-continental waters, estuarine outlets and deep offshore waters to at least 700 meters (Last and Stevens, 1994). Two species of stingarees, *Urolophus cruciatus* and *Urolophus expansus* are commonly caught as bycatch off the southwestern coast of Victoria.

The life history of stingarees in Australia is poorly understood with only few published studies from the west coast of Australia (White, 1998; White et al., 2001) and Port Phillip Bay, Victoria (Edwards, 1980). In this paper we describe the diet, reproductive biology and age estimates of *U. cruciatus* and *U. expansus*

March and June 2001. These specimens were bycatch of commercial otter trawls (40 m foot-line; 90 mm or 110 mm stretch mesh) fished at depths between 198 and 324 m. Trawling was conducted at speeds between 2.8 and 3 knots with sweep lengths of 180 m.

Specimens were identified and blotted dry, weighed (± 1 mm) and total length, TL (± 1 mm), disc width, DW (± 1 mm), mouth length, ML (± 1 mm) and length of tail spine (± 1 mm) recorded according to Last and Stevens (1994).

Stomachs were removed, weighed and fullness assessed using a scale from 1–5 where 1 is empty and 5 is full. Stomachs were stored in 70% ethanol and examined later under a dissecting microscope. Prey items were identified to the lowest possible taxonomic level using Jones and Morgan (1994) and Edgar (2000). Victorian Museum staff assisted when identifications could not be made from available literature. Number of prey species and the number of individual prey items were recorded for each stomach. Diet data was analysed using the Frequency

of Occurrence ($F = (\frac{N}{T}) \times 100$) (Hyslop, 1980).

Where N is the number of stomachs containing a particular prey item and T is the total number of stomachs examined.

Analysis of similarities (ANOSIM, PRIMER v5.2.0) (Clarke and Gorley, 2001; Clarke and

MATERIALS AND METHODS

Sample collection and location

A total of 50 *U. cruciatus* and 93 *U. expansus* were collected from southwestern Victorian waters by commercial vessels targeting *Pterygotrigla polyomata* (latchet), *Neoplatycephalus aurimaculatus* (toothy flathead), *N. richardsoni* (tiger flathead) and *Nemadactylus macropterus* (jackass morwong) between

Gorley, 2001) was used to determine differences in diet between sex, species and size.

Reproductive development was assessed using the stages of Snelson et al. (1988). Female stages were; (1) immature, (2) developing, (3) mature, non-pregnant, (4) mature, pregnant, and (5) mature, post-partum. Male stages were; (1) immature, (2) maturing, virgin (3) mature, non-reproductive, and (4) mature, sexually active. Clasper length was measured to the nearest mm using callipers and clasper calcification recorded.

Vertebrae were used to obtain preliminary assessments of age. After dissection from specimens, vertebrae were immersed briefly in boiling water and cleaned of remnant connective tissue. Dried vertebrae were embedded in polyester/epoxy resin and 0.3 mm sections cut longitudinally using a diamond saw. Sections were viewed under a compound microscope at $\times 10$ magnification. If band definition was unclear sections were enhanced by emersion in methyl salicylate. Band counts were repeated three times, with 25% of the samples counted by an independent reader. Growth was described using the von Bertalanffy growth model (with t_0 constrained to 0) using Fisat II (Gayanilo and Pauly, 2001). Vertebrae were aged 'blind' and re-analysed twice for standardisation and accuracy with reader precision assessed using the Index of Average Percent Error (Beamish, 1981).

$$\text{IAPE} = \frac{100}{N} \sum_{j=1}^N \left(\frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - X_j|}{X_j} \right)$$

Where

N = number of fish aged

R = the number of age determinations for each fish

X_{ij} = the i th determination for the i th fish

X_j = the average estimated age of the i th fish

RESULTS

Dietary composition of *U. cruciatus* and *U. expansus*

Prey species found in the stomachs of *U. cruciatus* and *U. expansus* represented six phyla and 15 families (Table 1). Crustaceans dominated the diet of both species, isopods *Natanolaua woodjonesi* and *N. wowine* being the most common. Polychaetes had a higher frequency of occurrence in *U. cruciatus* while greater numbers of polychaetes occurred in the diet of *U. expansus*. Adult insects (unidentified

due to being partly digested) were found in the stomachs of both species; however these results are considered an anomaly and may have been consumed by the animals while on the sorting tables of trawlers. Twenty-three prey species were present in the stomachs of both *U. cruciatus* and *U. expansus* with eleven being common to both species (Table 1).

Variations of diet between size class, sex and species

Two distinct cluster patterns and two outliers (Fig. 1) were evident in the hierarchical cluster analysis of the diet of *U. expansus* and *U. cruciatus*. The stress factor for the MDS was 0.09 suggesting a low risk of drawing false inferences (Clarke, 1993). No consistent patterns in clustering by sex or species were evident. However, distinct length-based grouping occurred with *U. expansus* differing amongst the 151–200 mm (C), 201–350 mm (A), and 351–500 mm TL (B) size classes and *U. cruciatus* differing amongst the 251–300 mm (A), 301–400 mm (B) and 401–450 mm TL (D) size classes.

Although ANOSIM demonstrated that each size class differed significantly ($R=0.555$), it also showed that both species were similar with an R-statistic value of 0.143 and a stronger correlation between all males and females ($R=-0.012$). Further analyses on male and female diet within size classes were combined because of the absence of differences in diet by sex. The R-statistic value for males and females of *U. expansus* had a close relationship of -0.031 while *U. cruciatus* followed a similar trend of $R=0.019$. A separate ANOSIM test on all males from both species showed a correlation between the two ($R=0.105$) while an individual test performed on females alone showed no similarities ($R=0.648$). Although there were no correlations in *U. expansus* size classes, *U. cruciatus* correlated well within its species with an R-statistic value of -0.158.

Age Estimates

A sample of 49 *U. cruciatus* and 87 *U. expansus* sections were examined under the microscope. An intra-reader precision test was performed on all readable vertebrae from both species. IAPE's for *U. cruciatus* and *U. expansus* were 7.51% and 6.02%, respectively, indicating high degrees of repeatability in the estimation of ages. von Bertalanffy growth parameters (Table 2) showed that female *U. expan-*

Phylum	Class/Order	Family	Species	<i>U. cruciatus</i>		<i>U. expansus</i>	
				%F	%N	%F	%N
Annelida	Polychaeta	Eunicidae	Total	52	20.37	26.88	32.95
			*Sp. 1	8	3.7	6.45	2.73
			*Sp. 2	8	1.65	8.60	1.93
			*Sp. 11	6	0.82	0	0
			*Sp. 23	0	0	3.23	0.64
		Flabelligeridae	*Sp. 10	12	4.73	2.15	4.66
		Pectinariidae	*Sp. 15	24	8.44	2.15	0.64
		Amphinomidae	*Sp. 19	0	0	12.9	22.35
		Scalibregmatidae	*Sp. 36	8	1.03	0	0
Nematoda			*Sp. 7	10	1.44	12.9	3.54
Priapulida		Priapulidae	Total	8	1.44	4.30	0.80
			*Sp. 16	8	1.44	2.15	0.32
			*Sp. 28	0	0	2.15	0.48
Crustacea			Total	80	76.13	73	65.12
	Isopoda	Cirolanidae	Total	50	54.11	45.16	46.3
			<i>Natatalana waadjanesi</i>	40	17.9	22.58	11.41
			<i>Natatalana wawine</i>	26	36.21	39.78	34.89
	Amphipoda	Phoxocephalidae	Total	42	9.05	21.5	9.65
			*Sp. 14	26	5.76	16.13	7.88
			<i>Brolgus tattersalli</i>	22	3.29	8.6	1.77
	Decapoda	Pasiphaeidae	Total	36	12.97	31.18	9.17
			<i>Leptachelia sydniensis</i> (shrimp)	22	10.91	6.45	2.25
		Erangonidae	*Sp. 32 (shrimp)	12	1.85	17.2	5.47
		Palinuridae	<i>Jasus sp. (juvenile)</i>	2	0.21	0	0
		Portunidae	*Sp. 27 (crab)	0	0	2.15	0.32
		Scallaridae	<i>Scyllarus crenatus</i> (slipper lobster)	0	0	8.60	1.13
Mollusca	Cephalapoda	Sepiolidae	<i>Euprymna tasmanica</i> (dumpling squid)	4	0.41	0	0
Insecta	Unknown	Unknown	*Sp. 38 (winged insect)	1.08	0.16	0	0
			*Sp. 37	0	0	2	0.21

Table 1. Prey items found in *Urolophus cruciatus* and *Urolophus expansus*. Frequency of occurrence (%F) and contribution by numbers (%N) * = Unidentified.

sus grow to a smaller size than males (due to absent mature female specimens the von Bertalanffy growth equation may not accurately represent growth in this species) and *U. cruciatus* females growing to a larger size than males. It should be noted that these age estimates are yet to be validated.

Reproduction

Onset of maturity for male *U. expansus* was at approximately 360 mm TL (Fig. 2a). Of the 79 male *U. expansus* examined, 16 were immature while 63 were mature. Insufficient samples of female *U. ex-*

pansus were obtained to allow assessment of onset of maturity.

Male *U. cruciatus* matured at 315 mm TL (Fig. 2b). Five *U. cruciatus* males were immature while 22 were mature. Female *U. cruciatus* matured at 320 mm TL (78% of maximum observed total body length). Seven *U. cruciatus* females were immature and 16 were mature.

Male *U. expansus* and *U. cruciatus* reached sexual maturity at the age of 7 years (360 mm TL) and 6 years (315 mm TL) respectively. Female *U. cruciatus* attained sexual maturity at the age of 6 years at approximately 320 mm TL. Insufficient *U. expansus* females were collected to determine age at sexual maturity.

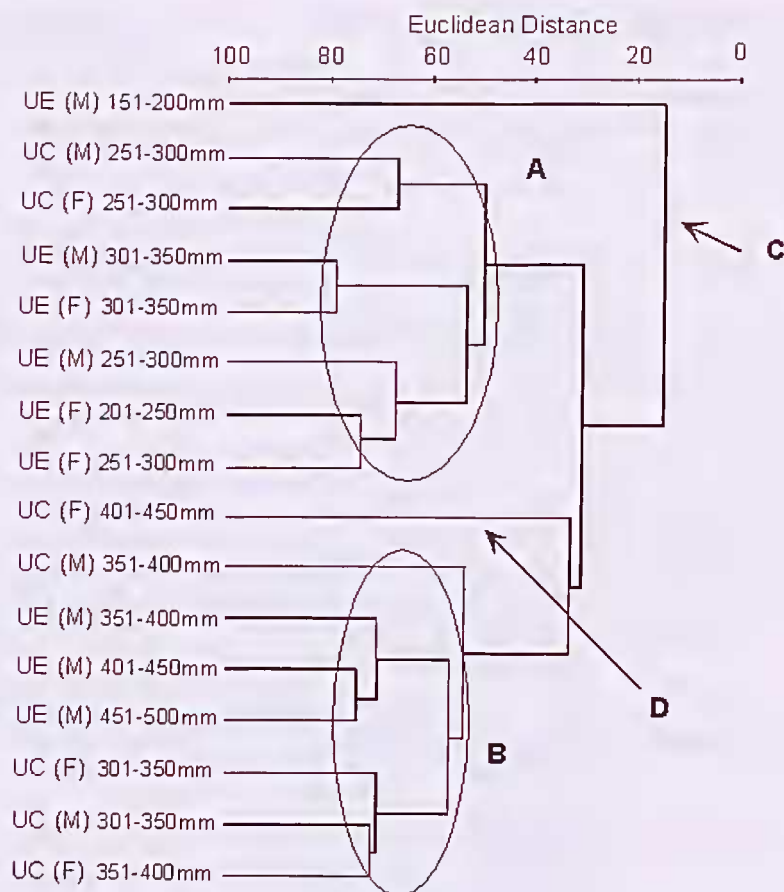


Fig. 1. Dendrogram of variations of diet for male (M) and female (F) *Urolophus expansus* (UE) and *Urolophus cruciatus* (UC) size classes (rounded to the nearest 10 mm) caught in south-eastern Australian waters.

Macroscopic Stages of Gonads

Urolophus expansus females were either stage 1 or 2 (immature with no ovarian development or very small ovaries). No specimens larger than 350 mm TL were collected. Over 50% of female *U. cruciatus* had stage 3 gonads, (mature but not pregnant). Two female specimens (378 mm and 335 mm TL) possessed two pups. Male and female pups had a mean total length of 114 mm and 105 mm, respectively and mean weight of 14.5 g and 10.8 g, respectively. One female had dark trophonemata and a flaccid/enlarged uterus suggesting it had recently given birth.

Most *U. expansus* males had stage 3 gonads (mature but non-reproductive) or stage 4 gonads (mature and sexually active). Males up to 310–350 mm TL were either stage one or stage two (still maturing). *U. cruciatus* males matured earlier

than *U. expansus* males as all animals were a stage 3 or 4 in the 310–350 mm TL size range.

DISCUSSION

Dietary Analysis

Urolophus cruciatus and *U. expansus* fed mostly on benthic organisms inhabiting sand and reef topography. The presence of coral, sponge and grit in their stomachs, combined with their mouth structure and morphology, suggests that these two stingarees are non-selective benthic feeders.

Crustaceans were the dominant prey in both species with isopods being the principle prey. Isopods can be parasitic or carnivorous (Hale, 1927–1929) with *Natatolana* species occurring in a range

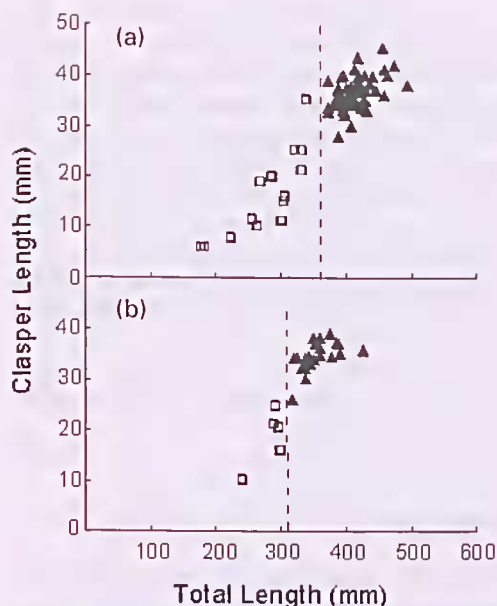


Fig. 2. Relationship between clasper length (mm) and total length (mm) for male a) *Urolophus expansus* (n=79) and b) *Urolophus cruciatus* (n=27) showing onset of maturity (represented by the dashed line) by clasper calcification and uncalcified caught from south-eastern Australian waters.

of sediments into which it burrows (Johansen and Brattegard, 1998), therefore it is easily preyed upon by bottom feeders. Most isopods examined were crushed and semi-digested indicating that they were presumably prey. Nematodes were found undigested in many stingaree stomachs. While there are many parasitic species in Australian temperate waters; most marine representatives live freely among the sediment and seaweed (Edgar 2000). These nematodes found were believed to be parasitic, as they were also present whole in the intestinal portion of the alimentary tract.

The presence of small numbers of benthic prey in the diet of *U. cruciatus* such as the dumpling squid (Family *Sepiolidae*) which buries itself into the sand (Norman and Reed 2000), sedentary polychaetes and amphipods suggests that *U. cruciatus* forages deeper into the sediment after prey. In contrast, *U. expansus* feeds more from the top sediments on epibenthic organisms including slipper lobsters (*Scyllarus crenatus*), crabs and more errant polychaetes that do not burrow as deeply into the sediments. ANOSIM showed extensive overlap in diet between *U. cruciatus* and *U. expansus*, despite only 11 of the 23 prey categories being common to both species.

There was no difference in diet between male and female of either species suggesting that there are no sexually based variations in foraging behaviour.

Urolophus expansus exhibited a great variation in diet with size, and fed on a range of prey species. Individuals from both species measuring between 150–300 mm TL preyed on small isopods, carid decapods and large numbers of amphipods (in different proportions). With increasing size, both species consumed a larger variety of prey with bigger isopods and amphipods ingested as well as prawns, slipper lobsters, priapulids and larger numbers of polychaetes. Isopods occurred frequently in the stomachs of all sizes of both species and the size of ingested isopods was positively correlated with stingaree size. Various studies (Andrews, 1988; Kohler and Fitzgerald, 1969; Platell et al., 1998) have shown that prey species are directly related to predator size. Platell et al. (1998) investigated the diet of *U. lobatus* and *U. paucimaculatus* off the south western coast of Australia in depths less than 35 m. It was found that *U. lobatus* and *U. paucimaculatus* initially fed mostly on amphipods, mysids and carid decapods. With increasing size, *U. lobatus* ingested teleosts and *U. paucimaculatus* preyed on polychaetes and penaeid decapods. Mysids were not found in the diets of *U. cruciatus* and *U. expansus*.

Species	Sex	L°	k
<i>Urolophus expansus</i>	Female	46.95 ± 34.6	0.21 ± 0.44
	Male	59.56 ± 4.29	0.14 ± 0.03
<i>Urolophus cruciatus</i>	Female	50.95 ± 4.26	0.18 ± 0.03
	Male	46.31 ± 4.17	0.21 ± 0.05

Table 2. von Bertalanffy parameter estimates (± 1 standard error) for male and female *Urolophus cruciatus* and *Urolophus expansus* collected from south-western Victoria. Data fitted using Fisat II (Gayaniilo and Pauly 2001) with t_0 constrained to 0.

which may be due to unavailability or selective feeding behaviours. Teleosts were not present in the diet of either species. Studies on *U. lobatus*, *U. paucimaculatus* and *Trygonoptera mucosa* in Australian waters also found that these species consumed teleosts in their adult stages (1998).

Our data suggests that *U. expansus* and *U. cruciatus* utilise similar habitats consuming 11 of the same organisms but in different proportions. The reason why only 11 of the specimens preyed upon were similar could be due to their different foraging techniques and mouth structure as discussed earlier.

REPRODUCTION

Size at Sexual Maturity

Based on elasper size and calcification, male *U. expansus* and *U. cruciatus* matured at 360 mm and 315 mm TL, respectively. Last and Stevens (1994) recorded the smallest mature *U. expansus* and *U. cruciatus* males to be 410 mm and 250 mm TL, respectively. Variations with Last and Stevens (1994) may be due to different sample sizes and population differences. With a larger sample size the estimated onset of maturity may be earlier than stated.

Urolophus cruciatus females matured at 320 mm TL. Only immature female *U. expansus* were examined and so length at maturity could not be determined.

From preliminary age estimates, the youngest pregnant female and mature male *U. cruciatus* were 6+ years. The youngest mature *U. expansus* male was 7+ years.

Gestation Period

Urolophids are viviparous (trochophore) giving birth to live young (Campbell, 1996). Gestation period was difficult to determine in both species because of the lack of mature, pregnant and post-partum animals. Female pups from *U. cruciatus* measured 103 mm TL and 107 mm TL with males having a total length of 111 mm and 117 mm. The birth size is unknown for these species though it is presumed that these pups were close to being released as they were well developed. The brown stingaree (*Urolophus westraliensis*) and Coral Sea stingaree (*Urolophus* sp. B) pups are born at total lengths of 100 mm (Last and Stevens 1994). Both *U. westraliensis* and *U. sp. B*

(Last and Stevens 1994) are tropical stingarees with males maturing at 240 mm and 230 mm TL respectively, a smaller size than *U. cruciatus* and *U. expansus*, suggesting a smaller length at birth.

As a generalization, stingarees have litters of 2–4 pups that take about 3 months to gestate (Last and Stevens, 1994), although White et al. (2001) found that *U. lobatus* in western Australia took 10 months to gestate. Since *U. cruciatus* and *U. expansus* are also found in Australian waters gestation could also be longer than 3 months. If gestation is longer than 3 months and litters are only 2–4 (a low fecundity) with survival rates unknown productivity of these species could be extremely low and commercial exploitation very dangerous.

Age and Growth

Elasmobranchs cannot at present be aged using traditional fish ageing methods, since they lack the necessary calcified structures i.e. otoliths and scales. Consequently spines and vertebral centra are commonly used for age and growth studies (Cailliet et al., 1986; McEachran et al., 1976). Validation was not carried out in this study due to small sample sizes. For the preliminary data required in this study it is presumed that growth bands were deposited annually, similar to other ageing studies of stingarees (White et al., 2001).

CONCLUSIONS

It was found that *U. cruciatus* and *U. expansus* are non-selective benthic feeders. Both species feed predominantly on the same organisms with isopods being the major prey item. No differences were found in the diet between males and females of the same species; however it was shown that diet composition and make-up varied with size. Diet variation occurred between the different size classes with smaller animals consuming smaller and fewer prey items. A greater variety of prey items became important in larger individuals.

This study has shown that *U. expansus* males mature at 7 years (36 cm TL, 24.7 cm DW) while female maturity could not be determined due to insufficient data. Both *U. cruciatus* sexes mature at 6 years though females mature at 31.5 cm TL and males at 32 cm TL. Disc width at maturity was 21.3 cm for both sexes.

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